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Influence of ionic strength and organic modifier concentrations on characterization of aquatic fulvic and humic acids by high-performance size-exclusion chromatography

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Abstract

Aquatic fulvic acid (FA) and humic acid (HA) were characterized by an aqueous high-performance size-exclusion chromatography (HPSEC) using a hydrophilic polymeric stationary phase and an aqueous eluent at neutral pH and low-ionic strength (5 mM Na_2HPO_4; final ionic strength, 13 mM). Employed HPSEC showed low sensitivity of FA to variations in ionic strength (13 and 100 mM) and contents of organic modifier (0.1 or 40% methanol) in aqueous eluent. Under these analytical conditions, peak maxima of relative UV signals versus molecular mass (M_r) defined as M'_p and peak maxima of relative mass concentrations versus M_r defined as M_p of FA were shown to be located at 548–690 and 500, respectively. Organic modifier concentrations of 40% methanol in aqueous eluent enabled not only analysis of FA, but also analysis of some aquatic HA by HPSEC. Analysis showed M'_p and M_p values of aquatic HAs around 1000 and 600, respectively. Measured molecular mass data of FA were found to be consistent with the recently published data describing low molecular masses of FA. Results recommend the use of the described HPSEC as a simple, rapid, reproducible, low-cost method giving consistent molecular sizes/masses of FA and some aquatic HAs.
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Keywords: Ionic strength; Mobile phase composition; Molecular mass; Fulvic acids; Humic acids; Humic substances

1. Introduction

The ability of humic substances (HSs), e.g. to inactivate various pesticides and other organic pollutants via complexation/copolymerization [1], to influence transport processes of organic and inorganic pollutants [2], as well as to lower bio-availability of harmful heavy metals via complexation [3] led to world-wide interest in research with these substances. For this purpose, realistic molecular masses of HSs are often required for the modeling and assessment of organic carrier-mediated migration of heavy metals as well

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as assessment of toxicological risk potential of heavy metals in terrestrial and aquatic ecosystems (cf. [4]).

In the past, various analytical methods were developed and used to characterize the HS components fulvic acid (FA) and humic acid (HA) (cf. [5]). Molecular masses/sizes of HSs were often estimated in the past using various analytical methods. One of the most popular methods is the analysis by size-exclusion chromatography (SEC; cf. [6,7]) and/or HPSEC (cf. [8–10]). Due to easiness and rapidness HPSEC was often used for the analysis of HSs, as this method is also thought to deliver molecular size distributions in response to UV signal. However, analysis of HSs by SEC or HPSEC is influenced by many parameters, as for example, type of column gel bed (stationary phase), calibration standards, eluent, ionic strength and organic modifier. Mostly HPSEC analysis of HSs was performed on silica-based stationary phase being very sensitive to variations in ionic strength and organic modifiers. Due to strong influences of slopes of calibration curves on relationships between molecular masses and hydrodynamic sizes of calibration standards, molecular masses of HSs determined were controversial in the past [11]. The hydrodynamic size of HSs also decreased as the pH of the aqueous eluent was lowered to pH 5 [12]. Addition of a small amount of $CaCl_2$ can reduce the hydrodynamic size while inducing the formation of associations without substantial aggregation [12]. The increment of ionic strength by NaCl in the eluent led to a shift of lower molecular size components (100–1000) to higher masses (1000–3000) while analyzing HSs by HPSEC, apparently due to aggregation [13]. A loosely bound self-association, micelle-like conformation of relatively small molecules of HSs in solutions similar to aggregation was also reported [14,15]. Piccolo et al. [14] and Conte and Piccolo [15] described a large shift from high to lower molecular size of HSs when HSs were pre-treated with organic acids.

HPSEC column was mostly calibrated in the past using, e.g. polystyrene sulfonate, dextran, polyacrylic acid, globular protein [6–10] as molecular size standards, mostly overestimating molecular masses of HSs. Due to intrinsic polyelectrolytic properties [16], aggregation-tendency [13–15] of HSs and dependence of their analysis on ionic strength and hydrophobicity, a rapid, reproducible, well calibrated HPSEC method with high recovery rate is required to handle a large number of samples. It should exhibit low dependence on ionic strength and hydrophobicity of the eluent. To achieve this goal, a careful selection of HPSEC parameters (e.g. gel bed, eluent, ionic strength, organic modifier, calibration standards) is to be developed for the analysis of HSs.

Previously, we published first results in brief [4] about a new application of a HPSEC method [17] for the molecular size/mass analysis of FA. Here, we describe in detail selection of carboxylic acid standards, influence of ionic strengths and organic modifiers on the molecular size/mass distributions of FA and HA by HPSEC. The suitability and reproducibility of this method at neutral pH for the rapid molecular size/mass analysis of FA and some HA are shown. Additionally, the method for conversion of HPSEC data of HSs into molecular mass distribution (MMD) data is presented.

2. Experimental

2.1. Chemicals, solvents and standards

All chemicals and solvents used in the study were of either analytical or HPLC grade and obtained from commercial sources (Merck, Fluka, Sigma, Aldrich and Riedel-de Haën, Germany). Polyacrylic acid and Suwannee River HSs (IHSS FA and HA) standards were purchased from Polymer Science Labs. (The Netherlands) and International Humic Substances Society (St. Paul, USA), respectively. Polystyrene sulfonate standards were obtained from Macherey Nagel (Germany).

2.2. Sampling and characterization of humic substances (HSs)

FA and HA were isolated from water of two peat bogs in the Dachauer Moos near Munich (FA 1.1, HA 2.3.1) and Gorleben aquifer [Gohy-573(HA)]. Water was filtered in the field (0.3 μm inorganic filter) and the HSs were isolated in the laboratory by adsorption on XAD-8 resin (Rohm and Haas) at pH 2. Following elution of the HSs at pH 13 and acidification to pH 1, HA (insoluble at pH 1) was separated from the FA (soluble at pH 1) by centrifugation. After purification by repeated adsorption/elution, the FA was freeze-dried either without or after desalting on a

cation-exchange resin AG MP-50 (Bio-Rad) column. The composition of the extracted HSs was about 90% FA (FA 1.1) and 10% HA, or 24% FA and 76% HA [Gohy-573(HA)]. The details are given elsewhere [18,19]. Isolated HSs were characterized by elemental analysis, proton-exchange capacity analysis, isotope analysis, copper complexation properties, ^{1}H and ^{13}C NMR spectroscopy and fluorescence spectrometry [4,18–21].

2.3. Determination of molecular sizes of fulvic acid

The molecular sizes of FAs were determined using HPSEC. The results were further verified by aqueous SEC.

2.3.1. HPSEC

The method was previously developed for the analysis of water-soluble polymeric substances of xenobiotic degradations using polystyrene sulfonate (PSS) as molecular mass calibration standards on a polymeric hydrophilic gel [17] with high tolerances of salt concentrations up to 8 M and polar organic solvents up to 100% (Macherey Nagel). In order to avoid overestimation of molecular sizes of the carboxylic analyte FA, instead of frequently used PSS standards various aliphatic and aromatic carboxylic acids including polyacrylic acid standards (Polymer Labs.) were used for molecular size calibration of the column after each change of eluent (see below). The discrete carboxylic compounds malic acid (M_r, 134.09), benzene-1,3-dicarboxylic acid (166.13), citric acid (192.13), benzene-1,2,4,5-tetracarboxylic acid (254.15), EDTA (292.25), DETPA (393.35), as well as polyacrylic acid standards M_p 1250 and 2925 were used. Effects of two different ionic strengths (13 and 100 mM) on molecular size/mass distributions of HPSEC data were investigated. Additionally, contents of the organic modifier methanol in the eluents were varied from 0.1 to 60%. Thus, the following aqueous eluents were used: (1) 5 mM $Na_2HPO_4 + 0.1\%$ methanol with a final ionic strength of 13 mM (pH 7.0, adjusted with 3 N H_3PO_4), (2) 5 mM Na_2HPO_4 + 0.1% methanol with a final ionic strength of 100 mM (pH 7.0, adjusted with 3 N H_3PO_4 and NaCl), and (3) 5 mM Na_2HPO_4 (pH 7.0; final ionic strength, 13 mM) + 40% methanol. The total permeation volume (V_p) was determined with KNO_3 and the void volume (V_0) either with blue dextran (M_r, ca. 2×10^6) or with PSS standard (M_p = 780,000) [17]. Each analysis was run at least in duplicates at a flow rate of 0.5 ml/min at a constant temperature of 30 °C. FA or HA was dissolved in the respective eluent and usually a volume of 20 μl containing 0.4–1 μg sample was injected for analysis. The UV data (λ = 240 nm) were acquired on-line using two HPLC systems: (1) a Hewlett-Packard HPLC system (Model HP 1090) connected to a Hewlett-Packard photodiode array detection (DAD) system and (2) a Shimadzu Inert HPLC system (Model LC-10Ai CE, Shimadzu, Germany) connected to a Shimadzu DAD system, whereby the data from both systems were processed by Shimadzu GPC software, Version 1.1, using a personal computer. Calibration curves with 95% confidence limits were calculated by regression analysis from triplicate runs of standards (see above) after each change of eluent.

2.3.2. Aqueous SEC

The SEC system (Pharmacia) consisted of a column (70 cm × 1.6 cm i.d.), a peristaltic pump (P1), a variable-wavelength monitor (Unicord VW 2251) and a fraction collector. The column was packed with Sephadex G-25S (superfine) gel with a size-exclusion limit of 5000 (Pharmacia). The eluent was usually 10 mM KCl and the ionic strength of the eluent was further increased to 100 mM KCl in order to assess the effects of ionic strength on molecular size/mass distributions of HSs. The flow rate of the eluents was 0.5 ml/min. The absorbance of the samples and standards was recorded at 195 nm. The total volume of the column was 120 ml. The void volume (V_0) 44.8 ml and the total permeation volume (V_p) 100 ml were determined using blue dextran (M_r, ca. 2×10^6) and acetone (M_r, 58.08), respectively. For molecular size calibration, polyethylene glycols (PEGs) of various molecular masses (M_p: 194, 200, 300, 400, 440, 600, 750, 900, 1080, 1470, 1500, 2000, 3000; Macherey-Nagel and Merck) were used as standards. The reproducibility of the calibration values was in general ±1%. Usually, 18.5–185 μg of FA was dissolved in the eluent and a sample volume of 500 μl was injected for analysis. Calibration curves with 95% confidence limits were determined by regression analysis from K_d-values (cf. [17]) calculated from triplicate runs using PEGs.

2.4. Calculation of molecular mass distributions

For verification of results measured by different systems, relative mass concentrations versus molecular mass (M_r) of HSs were calculated from relative UV signals and depicted against M_r (see results). Relative UV signals versus M_r were corrected according to Eq. (1), where the SEC and HPSEC data were transformed to calculate the differentials to give molecular mass distributions (MMDs) corresponding to $W(M_i)$ (cf. [22]):

$$W(M_i) = \left| \frac{H_i}{\sum_{i=1}^{i=n} H_i} \left(\frac{\mathrm{d}t_R}{\mathrm{d}M} \right)_i \right| \quad (1)$$

Here, H_i is the ith relative signal, t_R the retention time (min) and M the uncorrected molecular mass at each data point from calibration curves using each t_R (see above). For better verifications, the calculated values were re-scaled after $\mathrm{d}t_R/\mathrm{d}M$ corrections relative to the maximum mass concentration. Peak maxima of relative UV signals versus M_r (Da) and relative mass concentrations versus M_r are defined as M'_p and M_p, respectively.

2.5. Determination of HSs recovery using HPSEC

Recovery (%) of FA and HA on the HPSEC column depending on methanol concentrations in 5 mM Na_2HPO_4 (pH 7.0; final ionic strength, 13 mM) was related to the integrated signal of HSs at $\lambda = 240$ nm without the column. Here, 100% recovery is defined as the peak area (integrated UV signal) of the same concentration of the same type of FA or HA without the column.

3. Results

3.1. Molecular mass/size distribution of fulvic acid

The calibration curves $\log M_r$ versus t_R (min) for HPSEC (various eluents, see Section 2) and SEC (eluent: 10 mM KCl) systems were calculated by regression analysis. The standard deviation, precision and accuracy of the HPSEC system were <0.2, <2.9 and <7.5%, respectively [17]. As t_R (or elution volume) is dependent on hydrodynamic size of analytes during SEC or HPSEC, first M_r calculations of FA from calibration curves reflect more the character of molecular size than molecular mass. Correspondence of molecular size to molecular mass may show the approximation of M_r to absolute molecular mass.

The original UV signals of FA versus t_R measured by HPSEC and aqueous SEC are transformed into relative UV signals versus M_r and further into relative mass concentrations versus M_r using Eq. (1), whereby relative mass concentrations versus M_r are corrected for equidistant $\mathrm{d}t_R$ and $\mathrm{d}M$ values. The distributions of relative UV signals versus M_r and relative mass concentrations versus M_r of FA obtained by HPSEC (FA 1.1, IHSS FA) and aqueous SEC (FA 1.1) are depicted in Fig. 1. The relative UV signals versus M_r showed significant higher peak maxima (M'_p) at 605−850 as compared to peak values (M_p) at 483–581 calculated from MMDs (see Section 2.3). The calculated MMDs from HPSEC and aqueous SEC data of FA exhibited a similar distribution pattern, but a significant left shift of molecular masses in the direction of lower M_r range in comparison to corresponding relative UV signals versus M_r (Fig. 1).

Fig. 2 shows the influence of ionic strengths and hydrophobicity in various eluent compositions on the relative UV signals versus M_r of FA (FA 1.1) following each new calibration of the HPSEC column after each change of eluent. M'_p of FAs were located between 548 and 690 despite the variations of ionic strengths and contents of organic modifier (methanol) in eluent during HPSEC analysis. Only minor influences of different ionic strength (13 and 100 mM) at neutral pH and organic modifier contents (0.1 and 40%) in eluent on the distributions of relative UV signals versus M_r for FA were observed. Similarly, 100 mM ionic strength instead of 10 mM ionic strength (adjusted with KCl) in the aqueous eluent of SEC system did not affect significantly the respective chromatograms of FAs.

3.2. Dependence of recovery of HSs on methanol contents

The elution pattern of FA (FA 1.1) with different methanol contents (5, 25, 50 and 60%) in the eluent exhibited only a small shift (Fig. 3(a)), but in contrast to that, HA [(Gohy-573(HA)] showed a shift as well as an increasing tailing with decreasing methanol contents (Fig. 3(b)). Recovery of FA was up to 96% using HPSEC system (Fig. 3(c)) and about 92% using SEC

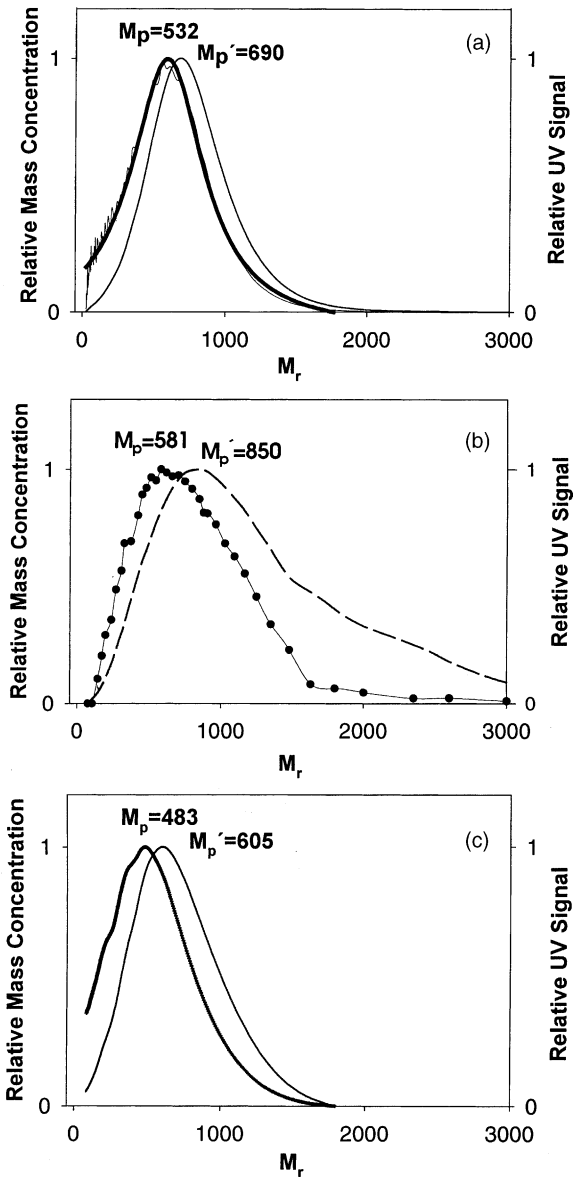

Fig. 1. Relative UV signals (narrow or dashed lines) and molecular mass distributions (MMDs) (bold lines and solid circles, calculated according to Eq. (1)) vs. molecular mass (M_r) of FAs calculated from data measured by: (a) HPSEC (FA 1.1), calibration curve $\log M_r = 11.451346 - 0.429447 R_t$, $r^2 = 0.86$; (b) aqueous SEC, calibration curve $\log M_r = 3.415271 - 1.745347 K_d$, $r^2 = 0.95$, and (c) HPSEC (IHSS FA), calibration curve $\log M_r = 8.133563 - 0.294968\, R_t$, $r^2 = 0.82$. Note: R_t = retention time.

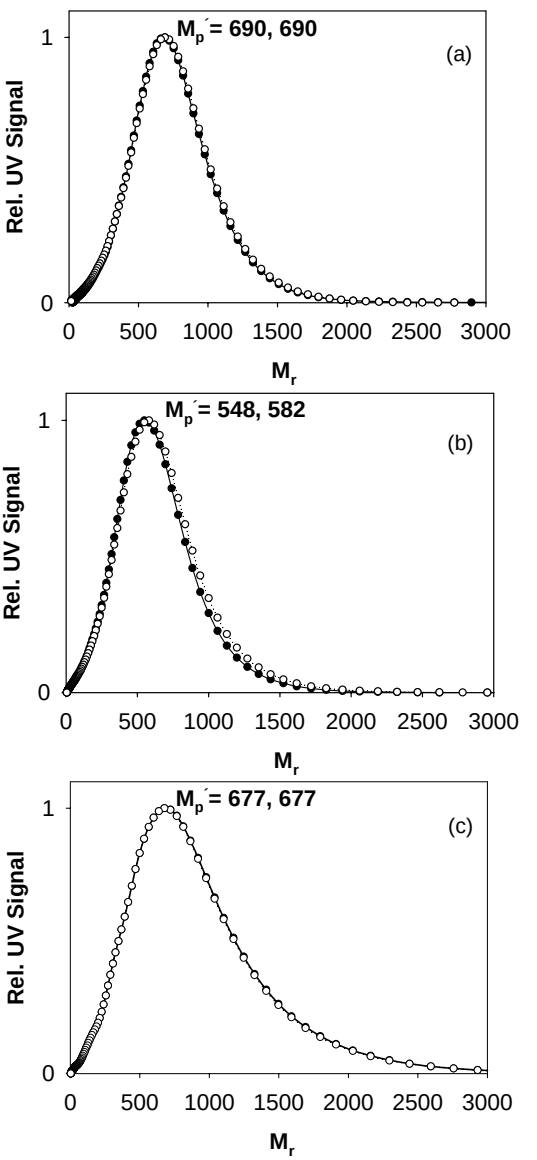

Fig. 2. Relative UV signal vs. M_r of FA (FA 1.1) by HPSEC depending on ionic strength: (a) eluent 1 (ionic strength, 13 mM), calibration curve is the same as given in Fig. 1(c); (b) eluent 2 (ionic strength, 100 mM), calibration curve $\log M_r = 10.567760 - 0.408491 R_t$, $r^2 = 0.68$; and (c) eluent 3 (ionic strength, 13 mM), 40% methanol (see Section 2), calibration curve $\log M_r = 10.133505 - 0.414220 R_t$, $r^2 = 0.92$. Note high reproducibility by two independent measurements (solid and open circles) and similar positions of M_p'.

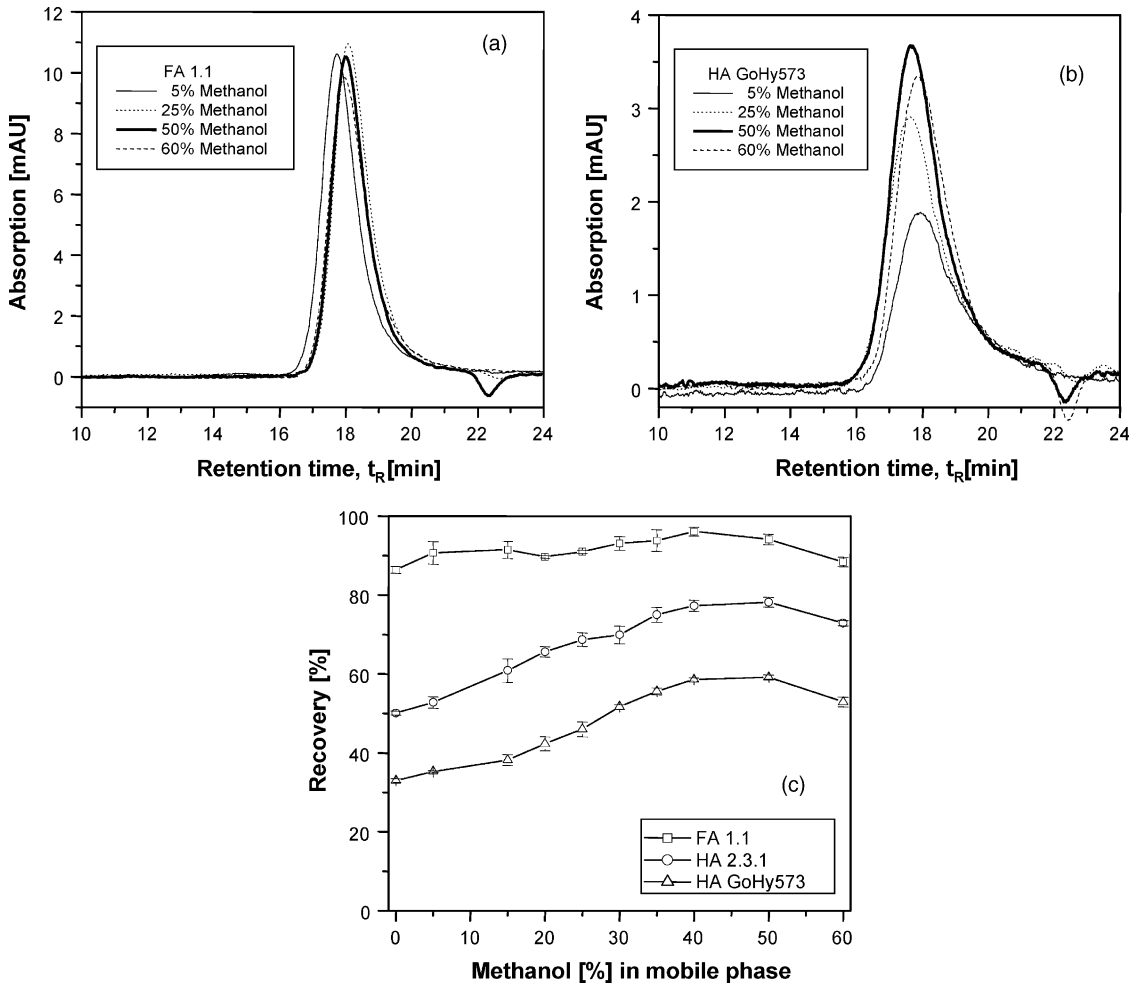

Fig. 3. Effects of methanol on the HPSEC chromatogram of: (a) FA (FA 1.1) depending on methanol contents in 5 mM Na_2HPO_4 buffer (pH 7.0; ionic strength, 13 mM); (b) HA [Gohy-573(HA)] depending on methanol contents in 5 mM Na_2HPO_4 (pH 7.0; ionic strength, 13 mM); and (c) recovery (%) of FA (FA 1.1) and HA [HA 2.3.1, Gohy-573(HA)] on HPSEC column depending on methanol contents in 5 mM Na_2HPO_4 buffer (pH 7.0; ionic strength, 13 mM; see Section 2).

system. Depending on origin of aquatic HA, the recovery of HA on HPSEC differed significantly. Estimated recovery of HAs reached the maximum values (up to 80%) at 40−50% methanol with nearly linear increments depending on HA type (Fig. 3(c)).

3.3. Molecular size/mass distribution of humic acid

Fig. 4 shows relative UV signal versus M_r and MMD of aqueous HA (HA 2.3.1, IHSS HA) at recovery rates of max. 80% using 5 mM Na_2HPO_4 (pH 7.0; ionic strength, 13 mM) + 40% methanol as optimized eluent in HPSEC system. Peak maxima (M'_p) of relative UV signal versus M_r were located at 970 Da (HA 2.3.1) and 1090 Da (IHSS HA), and corresponding M_p values of MMDs (see Section 2.3) at 560 and 570, respectively.

4. Discussion

Physico-chemical properties of HSs, especially of HAs should be considered for their successful

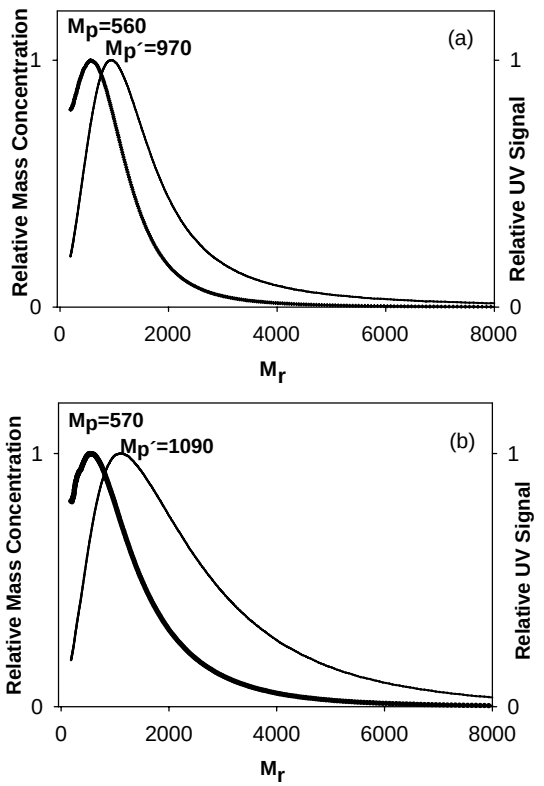

Fig. 4. Relative UV signals (narrow lines) and molecular mass distributions (MMDs; bold lines, calculated according to Eq. (1)) vs. molecular mass (M_r) of HAs calculated from data measured by HPSEC: (a) HA 2.3.1 and (b) IHSS HA at recovery rates of max. 80% using eluent 3 (see Fig. 3(c)). Calibration curve is the same as given in Fig. 2(c).

analysis. Generally, it is accepted that HSs have high cation-exchange capacity due to the presence of a large number of carboxyl and hydroxyl groups [23,24]. Coulombic (ion-exchange or ion-exclusion interactions) and Van der Waal's interactions including adsorption effects may occur between polyelectrolytic HSs and gel bed [5,6,9]. Repulsive and attracting electrostatic forces arising from pH-dependent dissociations of solute molecules, the presence of ionic species, ion-pairing agents, etc. as well as different ionic strengths may influence stretching of solutes and, as such hydrodynamic diameters (cf. [5]). Choice of stationary phases (gel beds) for HPSEC is an important aspect, as some gels may exhibit non-size-exclusion behavior requiring a careful adjustment of ionic strength, pH, buffers, and comparable standards to suppress the non-size-exclusion effects. Silica-based gels with hydrophilic surfaces for HPSEC analysis of HSs may bear a net negative charge due to the presence of underivatized silanol groups and, as such gives rise to exclusion of the anionic solutes [9]. Ionic interactions of, e.g. glyceropropylsilyl-bonded silica-based gels at neutral pH are almost completely neutralized with 83 mM ionic strengths and further more with 166 mM ionic strength [9]. In contrast, polymer-based gel beds with hydrophilic surfaces in our case do not possess underivatized silanol groups and, as such do not require high ionic strengths at optimum pH 7.0 (cf. [17]). Perminova [25] mentioned also later the non-size-exclusion effects of different pH values other than 7.0 on the SEC behavior of HSs. Calibration standards for HS analysis should be carboxylic or polycarboxylic acids in order to be comparable with polycarboxylic FAs and HAs to avoid overestimation of molecular sizes (cf. [4]) and deliver fast approximations of MMDs. Phosphate buffers rather than buffers with high complexing ability are preferable, as high complexing buffers may interact with, e.g. hydroxyl groups of the analytes and, as such may change physical–chemical properties, e.g. hydrodynamic sizes of analytes. Hydrophobicity of eluent should be adjusted to that of solutes by addition of organic modifier [17] in order to achieve high recovery rate of HSs by suppression of hydrophobic adsorptions. These criteria were considered in the development and the optimization of HPSEC method described here for determinations of more realistic sizes/masses of HSs.

Results of HPSEC analysis of FA and HA using the same analytical set-up and eluents of various ionic strengths and/or organic modifier (methanol) contents are presented using UV detection system, as the UV signal intensity can be related at least to a first approximation to the amount of HSs (cf. [5]).

Based on the ratio of $V_p/(V_p - V_0)$, which is a characteristic constant of a SEC or HPSEC system, our HPSEC system showed about 21 and 35% higher separation range than those of the SEC system on Sephadex G-25S (superfine) (see Section 2) and on Toyopearl HW-50S [7], respectively. However, due to the high mass transfer rate, the actual SEC resolution power of the HPSEC system is many times higher than that of any corresponding SEC system. The comparison of resolution of a HPSEC or SEC system using our method is an addition to the calculation of specific

resolution R_{sp} of a SEC column for two polymers, e.g. calibration standards, that differ by a decade of M_r [26].

MMDs of FAs obtained by HPSEC on the basis of relative mass (M_r) concentrations versus M_r using Eq. (1) were shown to be similar to MMDs calculated from time-of-flight (TOF) secondary ion mass spectrometry (SIMS) [4,11]. The M_p values fall fully, e.g. in the mass range of $200 \leq m/z \leq 800$ shown by FT–ICR (ion-cyclotron resonance) mass spectra of aquatic FAs in LD (laser desorption) and electrospray ionization (ESI) modes [27] and near to MMD maximum at M_r 450 using electrospray-quadrupole time-of-flight mass spectrometry [28]. In contrast to FT–ICR–MS, our data showed that the actual upper range of molecular mass of FA might be as high as 2000.

The employed HPSEC system using hydrophilic polymeric phase was relatively insensitive to changes of ionic strengths investigated at 13 and 100 mM. Only minor effects of increasing hydrophobicity (methanol contents) on peak maxima ($M'_p = 548$–690) of FAs were detected. At 40% methanol contents the signal intensities were slightly stronger than at 0.1% methanol. M_p values of FA ranged between about 500 and 600, and the M'_p values were located well below 1000 Da independent of ionic strengths and methanol contents in HPSEC analysis.

The calibration curves log (hydrodynamic radius) versus log (molecular mass) of different standards, e.g. globular proteins, PSS, dextran, showed different slopes resulting in systematic deviations depending on molecular mass [11]. Therefore, use of such calibration curves for analytes not located in the same slope may lead to erroneous estimations.

Due to high polarity of FA and low interactions of methanol with analyte and gel matrix at neutral pH (7.0), the recovery of FA by our new HPSEC was nearly independent of methanol contents ($\geq$0.1 and $\leq$60%) in 5 mM Na_2HPO_4 (pH 7.0; final ionic strength, 13 mM). However, the recovery of aquatic HAs by the same system was only about 30–80% depending not only on methanol contents in the eluent, but also on the type of HA, whereby 40% methanol in 5 mM Na_2HPO_4 (pH 7.0; final ionic strength, 13 mM; eluent 3) was found to be the best eluent for HPSEC analysis of aquatic HA separation. However, at this methanol concentration a maximum of 80% aquatic HAs was eluted from the HPSEC column depending on the origin of aquatic HA. It is to be mentioned that the recovery of HSs by HPSEC analysis is seldom given in the literature. The less the recovery of a particular HA by HPSEC or SEC analysis, the greater may be the error in the evaluated MMD. Due to signal shift depending on methanol contents, a new calibration of the HPSEC column with the same M_r standards at each change of eluent is required in order to assess the effects of methanol on MMDs of HSs. That is why using a new calibration curve of HPSEC column for the eluent 3, MMDs of eluted HAs (HA 2.3.1, IHSS HA) were calculated for max. 80% recovery. Under these conditions, M'_p values were located at 970 and 1090 Da, and MMDs of eluted HAs showed a range exceeding 3000. M_p values of the aquatic HAs derived from MMDs were found around 600. The eluent 3 was found to be suitable for HPSEC analysis of both FA and HA without changing the eluent. However, our method is not applicable for all types of HAs. High recovery of HAs and re-calculations of data according to Eq. (1) for MMD determinations are required while using such a system.

Our results are also consistent with special NMR measurements (NOE-effect enhancement) showing low molecular mass ($<\sim$2000) of HSs [29].

5. Conclusions

Our purpose was to present a fast, reliable HPSEC method based on hydrophilic polymeric gel material with relatively insignificant influence of ionic strength at neutral pH in obtaining representative M_r values of FAs in aqueous phase.

(1) All the analytical parameters of HPSEC system including calibration standards should be carefully chosen so that the analyte “FA” falls on the same slope of the calibration curve.
(2) Relatively insignificant effects of ionic strength and methanol as organic modifier on MMDs of FA were shown for our system. MMD peak maxima of FAs below 1000 were obtained by HPSEC and verified by SEC.
(3) Calculated MMDs support the lower range of published molecular mass data of HSs suggesting consistency of presented HPSEC method for the simple, economic, rapid and reliable

measurements of MMDs of FAs and some aquatic HAs.

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References

[1] J.-M. Bollag, Environ. Sci. Technol. 26 (1992) 1876.
[2] D. Klotz, D. Lazik, Isot. Environ. Health Stud. 31 (1995) 61.
[3] P. Zitko, W.V. Carson, W.G. Carson, Bull. Environ. Contam. Toxicol. 10 (1973) 265.
[4] M. Wolf, G. Teichmann, E. Hoque, W. Szymczak, W. Schimmack, Fresenius J. Anal. Chem. 363 (1999) 596.
[5] P. Janoš, J. Chromatogr. A 983 (2003) 1.
[6] R.S. Swift, A.M. Posner, J. Soil Sci. 22 (1971) 237.
[7] I.V. Perminova, F.H. Frimmel, D.V. Kovalevskii, G. Abbt-Braun, A.V. Kudryavtsev, S. Hesse, Water Res. 32 (1998) 872.
[8] Y.-P. Chin, G. Aiken, E. O'Loughlin, Environ. Sci. Technol. 28 (1994) 1853.
[9] J.P. Ralph, D.E.A. Catcheside, J. Chromatogr. A 724 (1996) 97.
[10] D. Tonelli, R. Seeber, C. Ciavatta, C. Gessa, Fresenius J. Anal. Chem. 359 (1997) 555.
[11] M. Wolf, G. Buckau, H. Geckeis, N.M. Thang, E. Hoque, W. Szymczak, J.-I. Kim, in: E.A. Ghabbour, G. Davis (Eds.), Humic Substances: Structures, Models and Functions, Royal Society of Chemistry, London, 2001, p. 51.
[12] M.E. Schimpf, M.P. Petteys, Colloids Surf. A 120 (1997) 87.
[13] Y. Kaneko, W. Agui, M. Abe, K. Ogino, Yukagaku 37 (1988) 108.
[14] A. Piccolo, S. Nardi, G. Concheri, Chemosphere 33 (1996) 595.
[15] P. Conte, A. Piccolo, Environ. Sci. Technol. 33 (1999) 1682.
[16] C.H. Lochmüller, S.S. Saavedra, Anal. Chem. 58 (1986) 1978.
[17] E. Hoque, J. Chromatogr. A 708 (1995) 273.
[18] M. Wolf, G. Teichmann, N. Hertkorn, Ph. Schmitt, M. Fischer, D. Jurrat, K.-P. Seiler, Annual Report 1995, GSF-Institute of Hydrology, Neuherberg, 1996, p. 187.
[19] J.I. Kim, G. Buckau, G.H. Li, H. Duschner, N. Psarros, Fresenius J. Anal. Chem. 338 (1990) 245.
[20] G. Buckau, Ph.D. Thesis, Faculty of Chemistry, FU Berlin, 1991.
[21] M. Wolf, D. Klotz, G. Teichmann, N. Hertkorn, W. Schimmack, in: Proceedings of the Series on Isotope Techniques in the Study of Environmental Change, IAEA, Vienna, 1998, p. 888.
[22] W. Szymczak, M. Wolf, K. Wittmaack, Acta Hydrochim. Hydrobiol. 28 (2000) 350.
[23] K.C. Beck, J.H. Reuter, E.M. Perdue, Geochim. Cosmochim. Acta 38 (1974) 341.
[24] R.L. Cook, C.H. Langford, Environ. Sci. Technol. 32 (1998) 719.
[25] I.V. Perminova, Soil Sci. 164 (1999) 834.
[26] S. Mori, H.G. Barth, Size Exclusion Chromatography, Springer, Berlin, 1999, p. 25.
[27] A. Fievre, T. Solouki, A.G. Marshall, W.T. Cooper, Energy Fuels 11 (1997) 554.
[28] G. Plancque, B. Amekraz, V. Moulin, P. Toulhoat, C. Moulin, Rapid Commun. Mass Spectrom. 15 (2001) 827.
[29] A.J. Simpson, W.L. Kingery, M.H.B. Hayes, M. Spraul, E. Humpfer, P. Dvortsak, R. Kerssebaum, M. Godejohann, M. Hofmann, Naturwissenschaften 89 (2002) 84.